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A study to find the utility of ZN staining in the diagnosis of confirmed cases of bone tuberculosis

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Introduction: As per the WHO guidelines, confirmed and definitive microbiological diagnosis of OATB is required before initiation of anti TB treatment. A study was conducted to find the utility of ZN staining in the diagnosis of confirmed cases of OATB cases. **Materials and Methods:** Study was conducted in the Department of Microbiology, GSL Medical College. Informed written consent was taken from all the volunteers. The study protocol was approved by the institutional ethics committee. Individuals aged ≥ 18 years who were clinically confirmed to be OATB were included in the study. Individuals with acute pyogenic osteoarticular infections were excluded. A simple FNAC technique was conducted in the outpatient unit for the collection of aspiration. Smears were prepared and stained by Ziehl Neelsen staining. Smear preparation and staining were done as per the RNTC guidelines. **Results:** During the study period 38 participants with suspected OATB were included. The smear positivity was 23 (60.5%). The male-female ratio was 1.08; statistically there was no significant difference (P = 0.753954). Age-wise maximum smear-positive cases were diagnosed in 58-67 years age group.**Conclusion:** In resource-limited areas, ZN staining is a good alternative for the diagnosis of OATB.

Keywords: Among, Smear, Tuberculosis, ZN staining

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To Browse

Introduction

Mycobacterium tuberculosis (MTB) complex is an acid-fast bacillus (AFB) is the causative agent of tuberculosis (TB). TB is a world pandemic and the leading cause of death worldwide [1]. Due to this high incidence and contributory factors such as poverty, TB is an Important reflector of the slow economic growth of developing countries, rendering loss of productive manpower and adverse economy. Nearly 90% of cases occur in low- and middle-income countries (LMICs) [2]. India is the highest TB burden country in the world, one-fifth of global TB burden, and the incidence is over 2 million annually [3,4].

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MTB can infect any organ in our body, but pulmonary TB (PT) is the commonest infection. Extra PT accounts for 15-20% of total TB cases. Among the total TB cases, 3% is an account for bone or osteoarticular TB (OATB) [5]. OATB not only leads to joint destruction but also spread the disease to adjustment soft tissues also. As per the WHO guidelines, confirmed and definitive microbiological diagnosis of OATB is required before initiation of anti-TB treatment due to its resembles with other infections [6]. Various techniques such as demonstration of AFB using ZN staining, fluorescent staining, culture on LJ media, rapid diagnostic methods such as demonstration of antibodies, molecular assays such as LPAand MODS are available for the detection of TB [7,8]. Among these techniques, demonstration of AFB by ZN staining is a simple, rapid, economical and highly specific staining method. With these, a study was conducted to find the utility of ZN staining in the diagnosis of confirmed cases of OATB cases.

Materials and Methods

Settings: It was a cross-sectional study, conducted in the Department of Microbiology, GSL Medical College.

Duration of study: The study was conducted from January 2018 to June 2019.

Sampling method: Random sampling was considered in this study.

Inclusion criteria: Individuals aged $\geq$ 18 years who were clinically confirmed to be OATB were included in the study.

Exclusion criteria: The individuals with acute pyogenic osteoarticular infections, individuals $\leq$ 18 years and who didn't submit the informed written consent were excluded from the study.

Sample size: All the individuals who satisfy the inclusion criteria during the study period were included in the study.

Ethical approval: Study protocol was approved by the institutional ethical committee.

From the study participants under sterile precautions, the sample was collected by aspiration using 20 gauge needle. It was a simple FNAC technique that was conducted in the outpatient unit. For accuracy in diagnosis, multiple samples were collected in the presence of orthopedics and a clinical pathologist. Then smears were prepared and Stained by ZiehlNeelsen staining. Smear preparation and staining were done as per the RNTC guidelines.

Smear preparation: A new unscratched slide was selected for smear preparation. Smear was prepared with a sterile loop. A good smear is spread evenly, over a size of 2 × 3 cm and is neither too thick nor too thin. This was allowed to air dry for15-30 min and fixed by passing it over a blue flame 3 – 4 times[9].

ZN staining: Smears, flooded with filtered 1% carbon fuchsin (CF) was heated until it was steaming and left to steam for5 min. After rinsing the slides with a gentle stream of water,25% sulphuric acid was used to de-colorize the smears for 2 – 4min and if necessary decolorization step may be repeated for another 1 – 3 min. The slides were rinsed as above and counterstained with 0.1% methylene blue for30 seconds. The slides were then washed, air-dried and examined under oil immersion [9].

Results

During the study period, 38 participants with suspected OATB were included. In this 21 (55%) were male and 17 (45%) were female, the male-female ratio was 1.23 (Table 1). When ZN staining results were considered, the smear positivity (SPT) was 23 (60.5%). In the smear-positive cases, 12 (31.6%) were male and 11 (29%) were female participants and the male-female ratio among the SPT cases was1.08; statistically there was no significant difference (P = 0.753954) (Table 1).

Table-1: Gender wise ZN results among the study participants; n (%).

Gender	ZN positive	ZN negative	Total
Male	12 (31.6)	9 (23.7)	21 (55)
Female	11 (29)	6 (15.7)	17 (45)
Total	23 (60.5)	15 (39.5)	38 (100)
Statistical analysis	Chi square = 0.0982; P = 0.753954. Statistically not significant.		

Age wise, 7.9% (3) were included in 18-27 years age group, 13.1% (5) in 28-37 years age group, 21% (8) in 38-47 years age group, 18.4% (7) in 48-57 years age group, 26.3% (10) in 58-67 years age group and 13.1% (5) in > 68 years age group. Age wise, in male ZN staining SPT was 2.6% (1) in 18-27 years age group, 2.6% (1) in 28-37 years age group, 7.8% (3) in 38-47 years age group, 2.6% (1) in 48-57 years age group, 10.5% (4) in 58-67 years age group and 5.2% (2) in > 68 years

Age group (Table 2). Whereas, among the female, age wise ZN SPT was 2.6% (1) in 18-27 years age group, 5.2% (2) in 28-37 years age group, 5.2% (2) in 38-47 years age group, 5.2% (2) in 48-57 years age group, 7.8% (3) in 58-67 years age group and 2.6% (1) in > 68 years age group (Table 2).

Table-2: Age-wise ZN results among the study participants; n (%).

Age	Male			Female		
	Positive	Negative	Total	Positive	Negative	Total
18 – 27	1 (2.6)	1 (2.6)	2 (5.2)	1 (2.6)	0	1 (2.6)
28 – 37	1 (2.6)	1 (2.6)	2 (5.2)	2 (5.2)	1 (2.6)	3 (7.8)
38 – 47	3 (7.8)	2 (5.2)	5 (13.1)	2 (5.2)	1 (2.6)	3 (7.8)
48 – 57	1 (2.6)	2 (5.2)	3 (7.8)	2 (5.2)	2 (5.2)	4 (10.4)
58 – 67	4 (10.5)	3 (7.8)	7 (18.4)	3 (7.8)	0	3 (7.8)
>68	2 (5.2)	0	2 (5.2)	1 (2.6)	2 (5.2)	3 (7.8)
Total	12 (31.6)	9 (23.7)	21 (55)	11 (28.9)	6 (15.8)	17 (45)

Discussion

Culture for MTB was considered to be the gold standard technique for the diagnosis of TB [10]. But the requirement of the prolonged-time period, infrastructure and manpower are the major issues. In tertiary health care setup, as a part of the training, paramedical students can be utilized for cultural facilities such as for media preparation, inoculation and so on. So due to the availability of various paramedical staff as well as paramedical student manpower is not an issue especially in tertiary health care setup. But accreditation from RNTCP is the major issue [11]. By considering the requirement of the prolonged-time period, in the current study, ZN staining was used for the microbiological diagnosis of OATB.HIV is another contributory factor of TB and it was reported that the chances of getting TB are 25 times among the TB HIV. Moreover, the diagnosis of HIV lung TB is also difficult due to the presence of very few bacilli. Whereas in this study, HIV status was not considered. In this study, with ZN staining, the SPT was 60.5%. Agarwal A et al. reported that with ZN staining, 86% of cases were diagnosed to be OATB [5]. Whereas the diagnostic utility for ZN was reported to be 75% by Arathi et al and 63% by Masood et al report [12,13]. Whereas the SPT was reported to be just 27% by Mousa et al [14]. When genders were considered, the SPT was 31.6% among the male and 29% among the female. The male-female ratio among the SPT cases was 1.08; statistically, there was no significant difference (P = 0.753954) (Table 1). As per Paloma Merino et al., Study, in extra PT, the male-female ratio was reported to be 1 [1]. Agarwal A et al. reported a male-female ratio of 3:7[5]. Whereas Beiner JM et al., reported male predominance in extra PT [15]. Age-wise, in this study, 38 – 67 years was considered to be the predominant age group with maximum (49.5%) TB cases. Q Sidek et al., also reported that the middle age group is the predominant group with a maximum number of TB cases [16]. This is due to a more outdoor activity so that infection can be transmitted easily. It is a common and burning issue in developing, high TB burden countries such as India [17]. In this study, 39.5% were negative for ZN staining. These 15 cases were clinically diagnosed to be OATB. At this point, we should consider one of the limitations of the technique. The presence of 10^4 bacilli/ml of sample is the minimum number to appreciate under ZN staining. So the sensitivity of the technique is limited. Culture on LJ media, molecular techniques should be the gold standard techniques [18, 19]. Due to the drawbacks such as the requirement of the prolonged-time period and high cost, respectively, these techniques were not considered in this study.

Limitations

Small sample size, short study period and considering a single technique for the diagnosis of OATB are the limitations of this research.Small sample size, short study period and considering a single technique for the diagnosis of OATB are the limitations of this research.

Conclusion

In resource-limited areas, ZN staining is a good alternative for the diagnosis of OATB.

What does this study add to the existing knowledge?

In spite of the limited sensitivity, ZN staining is a good alternative for the diagnosis of OATB.

Author's contributions

Dr. P Sunil Kumar: Literature survey, paper writing, data analysis

Dr. T Jaya Chandra: Complete idea, sample collection, benchwork, statistical analysis, paper writing.

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